Notes on the Design of Bioequivalence Study: Mebendazole

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.


Below, additional specific guidance is provided on the invited immediate release products containing mebendazole.

Pharmacokinetics of mebendazole

Following oral administration, <10% of the dose reaches the systemic circulation due to incomplete absorption and extensive pre-systemic metabolism (first-pass effect). However, as absorption does occur, systemic levels obtained following administration of a product need to be assessed for reasons of safety. To this end, pharmacokinetic bioequivalence trials are considered the best way to assess the biopharmaceutical quality of a multisource mebendazole product, thus avoiding the need to conduct full clinical trials to establish the safety and efficacy of the proposed mebendazole product.

Maximum plasma concentrations are generally seen 2 – 4 hours after administration. Administration with a high fat meal increases the bioavailability of mebendazole, but the overall effect of food on the amount of drug remaining in the gastrointestinal tract is not expected to be substantial.

Orally administered mebendazole is extensively metabolised primarily by the liver. Plasma concentrations of its major metabolites (amino and hydroxylated amino forms of mebendazole) are substantially higher than those of mebendazole. Mebendazole, the conjugated forms of mebendazole, and its metabolites likely undergo some degree of enterohepatic recirculation and are excreted in the urine and bile. The apparent elimination half-life after an oral dose ranges from 3 – 6 hours in most patients.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of mebendazole, the following guidance with regard to the study design should be taken into account:

**Design:** A single-dose replicate 4x2 cross-over design is recommended due to the high residual variability observed in 2x2 cross-over studies, to estimate intra-subject variability more accurately and to widen the acceptance range for C\text{max} and AUC\text{t} if the high variability of the comparator product is confirmed in a replicate design.

**Dose:** As the EoI includes only mebendazole 500 mg chewable tablets, this strength should be employed in the bioequivalence study. During treatment mebendazole tablets can be administered whole or chewed but, for the bioequivalence study, both products should be administered whole (unchewed).

**Fasted/fed:** The instructions for administration of mebendazole in its labeling indicates that mebendazole can be taken without regard to food intake. In the clinical studies conducted in pediatric patients with soil transmitted helminth infections, the majority of these patients were administered mebendazole tablets with food. In addition, as a high fat meal increases systemic exposure 2.6 fold for AUC and 4-fold for C\text{max}, a study in the fed state is recommended. However, a fasting state study may also be acceptable.
**Subjects**: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

**Parent or metabolite data for assessment of bioequivalence**: The parent drug is considered to best reflect the rate of release of the drug from the dosage form to the site of action in the lumen of the gastrointestinal tract. The data for the parent compound will be used to assess bioequivalence.

**Sample size**: The intra-subject variability (residual error) observed in a fasting state 2x2 cross-over study was 54.6% for AUC_T and 31.6% for C_max. Similarly, high intra-subject CV values have been observed in a 2x2 cross-over study in the fed state: 40.1% for C_max and 37.6% for AUC_T. However, in a 4x2 replicate cross-over design the intra-subject variability of the reference was reduced to 23.7% for C_max and 21.3% for AUC_T. Therefore, a replicate design is recommended. These data will facilitate the calculation of the sample size for the bioequivalence study with sufficient statistical power to demonstrate bioequivalence.

**Washout**: Taking into account the elimination half-life of approximately six hours, a washout period of seven days is sufficient to prevent carry over.

**Blood sampling**: The blood sampling for mebendazole should be intensive for the first six hours after administration to properly characterize the C_max of mebendazole, which is observed 4 hours after administration (range: 1 – 6 h). It is not necessary to take blood samples beyond 32 hours, e.g. pre-dose (0.00 hour) and 0.50, 1.00, 1.50, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.33, 5.67, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 18.00, 24.00 and 32.00 h post-dose.

**Analytical considerations**: Information currently available indicates that it is possible to measure mebendazole in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the C_max in most profiles of each formulation (test or comparator), e.g. LOQ of at least 0.25 ng/ml for a fasting study and 1 ng/ml for a fed study.

**Statistical considerations**: The data for mebendazole should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean AUC_T of the test to comparator product should be within 80.00 – 125.00%
- The 90% confidence interval of the relative mean C_max of the test to comparator product should be within 80.00 – 125.00%.

As it has been observed that the residual variability of C_max and/or AUC_T is high (CV>30%) in 2x2 cross-over studies, the applicant may design a replicate cross-over study to estimate intra-subject variability more accurately and to widen the acceptance range for C_max and AUC_T if the high variability of the comparator product is confirmed in a 4x2 replicate design. Please note that widening of the acceptance range for AUC_T for mebendazole will be accepted by PQT/MED. For more information on replicate study designs and widening of the acceptance range based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 6 TRS 1003. If widening of the acceptance range is planned for the AUC_T parameter, the principles described for C_max in Section 7.9.3 will apply and a four period, full replicate design study should be conducted to demonstrate bioequivalence, in order to assess the variability associated with each product.